

Effect of exenatide on postprandial glucose fluxes, lipolysis, and β -cell function in nondiabetic, morbidly obese patients.

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Running title: Metabolic effects of exenatide in obese non-diabetic subjects

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Abstract

Aims: To investigate the effect of exenatide on glucose disposal, insulin secretion, β -cell function, lipolysis, and hormone concentrations in non-diabetic, morbidly obese subjects under physiological conditions.

Materials and methods: Patients were assigned to exenatide 10 μ g twice daily (EXE, n=15) or control (CT, n=15) for 3 months. Patients received a meal test/tracer study (MTT) to measure endogenous glucose production (EGP), rate of oral glucose appearance (RaO), insulin secretion rate (ISR), β -cell function, hepatic (HIR) and adipose tissue insulin resistance (AT-IR) and insulin sensitivity (IS).

Results: Post-treatment the EXE group showed a significant reduction in body weight ($p<0.001$). The postmeal time-course of glucose, insulin, and ISR showed a lower peak between 60-180 min in phase with a reduction in RaO ($p<0.01$). After an initial similar suppression, EGP resumed at higher rates between 60-180 min ($p=0.02$) in EXE vs CT, while total RaO and EGP throughout the MTT were similar. In EXE, the postmeal glucagon, GLP1, and GIP responses were reduced ($p<0.05$). Fasting and postprandial lipolysis and β -cell function were unaltered by active treatment. HIR, AT-IR, and IS were all improved after exenatide treatment ($p<0.05$).

Conclusions: In morbidly obese non-diabetic subjects, exenatide causes weight loss, decreased postprandial glycaemia and glucagon response without changes in β -cell function. These effects are consequent upon delayed oral glucose appearance in the circulation. Exenatide treatment is also associated with an improvement of hepatic, adipose tissue, and whole body insulin sensitivity with no influence on postprandial lipolysis.

Introduction

GLP-1 analogues are used for the treatment of type 2 diabetes (T2D); more recently, due their effect on weight loss, their use has been extended to the treatment of nondiabetic obese patients [1-3]. Exenatide – a synthetic version of exendin-4 – mimics many of the effects of GLP-1. Thus, it potentiates glucose-induced insulin secretion and suppression of glucagon release in diabetic patients [4], and delays gastric emptying in a dose-dependent fashion. Chronic exenatide treatment improves all parameters of β -cell function in T2D patients treated with metformin or with metformin and a sulfonylurea [5] or without associated treatment [6]. Despite the increasing adoption of GLP-1 analogues for the treatment of obesity without diabetes [1, 3], their effect on β -cell function under physiologic circumstances has not been well characterised. Aulinger *et al.* [7] measured insulin secretion during clamped hyperglycaemia combined with graded GLP-1 infusion; they concluded that in young, healthy subjects, GLP-1-induced insulin secretion is proportional to insulin resistance but the sensitivity of the β -cell to GLP-1 varies widely and is independent of insulin sensitivity. In prediabetic patients, the GLP-1 analogue, liraglutide increased insulin secretion and β -cell sensitivity to intravenous glucose as compared with placebo [8]. Egan *et al.* [9] demonstrated an insulintropic effect of acute exendin-4 administration in both diabetic and non-diabetic patients using the hyperglycaemic clamp technique. However, in none of these studies was the effect of exenatide on parameters of β -cell function determined under conditions mimicking free living. Furthermore, few studies have analysed the impact of exenatide on glucose turnover under physiological conditions [10]. While GLP-1 does not affect peripheral insulin sensitivity (under euglycaemic hyperinsulinaemic clamp conditions) in either non-diabetic [11] or diabetic subjects [12], its effects on hepatic glucose handling are not clear since the hormone also suppresses glucagon release. In T2D patients, Cervera *et al.* [13] showed that an infusion of exenatide during a mixed meal decreases endogenous glucose production (EGP) and glucagon release through a reduction in the rate of oral glucose appearance. In the same study, the replacement of glucagon during exenatide infusion blunted

the suppressive effect of exenatide on EGP [13]. On the other hand, GLP-1 infusion inhibits EGP in lean healthy men independently of islet hormones under conditions of pancreatic clamp [14, 15]. Gastaldelli *et al.* [16] recently found that the acute administration of exenatide before an OGTT decreased EGP and increased hepatic glucose uptake of orally administered glucose despite lower insulin concentrations.

The effect of GLP-1 on lipolysis is controversial. *In vitro* studies documented a lipolytic effect of GLP-1 in isolated rat adipocytes [17] and 3T3-L1 adipocytes *via* cAMP signalling [18]. Exendin-4 has been reported to stimulate lipolysis in adipocytes from obese patients [19], while GLP-1 has been found to stimulate lipolysis in β -cells [20, 21]. In an *in vivo* study in healthy volunteers, GLP-1 infusion did not change whole-body lipolysis (assessed as the rate of labeled glycerol appearance) [15], whereas in patients with new diagnosis of diabetes or IGT, exenatide acutely enhanced the antilipolytic effect of insulin and reduced plasma FFA levels during OGTT [16]. In patients with nonalcoholic steato-hepatitis, liraglutide treatment decreased circulating free fatty acid (FFA) levels in the fasting state, and enhanced insulin-mediated suppression of lipolysis during an insulin clamp [22].

The aim of the current work was to explore the response of glucose and lipid metabolism and β -cell function to three months of exenatide treatment in non-diabetic, morbidly obese subjects studied under physiological conditions by using stable isotope tracers and mathematical modelling during a mixed meal test.

Methods

Subjects Thirty morbidly obese, nondiabetic subjects of either sex, aged between 30-60 years, with BMI $\geq 40 \text{ kg m}^{-2}$, eligible and wait-listed for bariatric surgery were assigned (in a 1:1 ratio) to maximal doses of exenatide (Byetta®, 10 μg twice daily) (15 subjects) or no-treatment (15 subjects) for 3 months in an unblinded fashion.

Exclusion criteria were: (a) type 2 diabetes mellitus (according to WHO criteria); (b) pregnancy; (c)

major psychiatric illness; (d) recent or current major eating disorders (anorexia nervosa or bulimia nervosa); (e) medical conditions requiring acute hospitalisation, (f) blindness, (g) severe medical conditions (liver cirrhosis, end-stage renal failure, malignancy, connective tissue diseases, endocrine diseases such as hypo- or hyperthyroidism) or illnesses such as chronic congestive heart failure, recent myocardial infarction or stroke, unstable angina pectoris, (h) treatment with pharmacologic agents known to affect carbohydrate homeostasis or antidepressant drugs (i) previous bariatric surgery, motility disorders of the gastrointestinal tract.

For the entire duration of the protocol, subjects were maintained on a diet with a caloric intake corresponding to their estimated resting metabolic rate by Harris-Benedict formula [23]. No patient was engaged in exercise programs beyond their habitual physical activity. All patients signed an informed, written consent prior to the study. The protocol was approved by the Institutional Ethics Committee.

Study design The study protocol included a screening period of 6-30 days before the baseline study and randomisation. During the screening period, inclusion and exclusion criteria were assessed through medical history and clinical laboratory testing performed in the previous 6 months. All concomitant or prior pharmacological therapies that might interfere with the results of the study were excluded. At baseline and following 3 months of treatment, all patients received a meal tolerance test (MTT) with the infusion of stable isotope tracers to measure rate of glucose appearance (Ra), glucose rate of disappearance (Rd) and glucose clearance (MCR_G) (by a primed constant infusion of 6,6-[²H₂]glucose), appearance of oral glucose (RaO), (by the addition of [U-¹³C₆]glucose to the oral glucose), and lipolysis (by a primed constant infusion of [⁵H₅]glycerol); endogenous glucose production (EGP) was then calculated as the difference between total and oral Ra.

Body composition was evaluated by electrical bioimpedance (TBF 300; Tanita, Tokyo, Japan) with standard formulas [24]. Fat mass was obtained as the difference between body weight and fat-free mass

Control visits during the treatment period were performed 30 and 60 days after randomisation. During these visits, clinical and laboratory measurements were repeated and treatment compliance and side effects

were monitored. At the final visit (day 90), the MTT and all clinical and laboratory measurements were repeated.

Mixed Meal Tolerance Test (MTT) and tracer administration After an overnight (12-hour) fast, subjects were admitted to our Clinical Research Unit at 8:00 am. After recording height, body weight, waist and hip circumferences, a polyethylene cannula was inserted into an antecubital vein for the infusion of all test substances. A second catheter was inserted retrogradely into an ipsilateral wrist vein on the dorsum of the hand for blood sampling while the hand was kept in a heated box at 65°C to achieve arterialisation of venous blood. Baseline blood samples were drawn to measure substrates and hormones.

A primed [$28 \mu\text{mol}\cdot\text{kg}^{-1}$]-constant [$0.28 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$] infusion of 6,6- $^2\text{H}_2$]glucose and a primed [$1.5 \mu\text{mol}\cdot\text{kg}^{-1}$]-constant [$0.1 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$] infusion of $^5\text{H}_5$]glycerol were started (at time -120 min) *via* the antecubital vein catheter and continued until the end of the study. During the last 20 min of the two-hour basal equilibration period (at times -20 , -10 , and 0 min), blood samples were obtained for the determination of substrates, hormones, and tracer enrichments (when isotopic steady state is achieved). After the basal equilibration period, the meal was consumed over a period of 10 min. The meal consisted of 75 g of glucose as an aqueous solution, 50 g of parmesan cheese and one 50-g egg, for a total of 585 kcal (18% protein, 31% fat and 51% carbohydrate). The glucose solution was enriched with U- $^{13}\text{C}_6$]glucose in order to trace oral glucose absorption. In the exenatide group, during the MTT performed following 3 months of treatment, exenatide was administered 20 min before the meal. Plasma samples for the determination of plasma glucose and hormone concentrations and glucose tracers enrichment were obtained at 15, 30, 45, 60, 90, 120, 150, 180, 240, 300 and 360 min after meal ingestion.

Data analysis EGP, RaO, and MCR_G were calculated from the time-course of the plasma tracer/tracee ratio of 6,6- $^2\text{H}_2$]glucose and U- $^{13}\text{C}_6$]glucose using non-steady state mathematical models, as previously reported [25, 26]. Glucose fluxes were normalized per kg of fat-free mass. Whole-body lipolysis was estimated from the glycerol rate of appearance (RaGly) [16, 22]. Insulin sensitivity was calculated as the ratio

of glucose clearance (MCR_G) to the mean plasma insulin concentration over corresponding time intervals (MCR_G/I) [26]. Insulin sensitivity during the entire MTT was also estimated using the Oral Glucose Insulin Sensitivity index, OGIS [27]. Since plasma insulin is a strong inhibitory stimulus for EGP and lipolysis, indexes of insulin resistance at the level of the liver (HIR) and of the adipose tissue (AT-IR) were obtained as the product of fasting EGP and fasting $RaGly$, respectively, and fasting plasma insulin concentration. The pre-hepatic insulin-to-glucagon molar concentration ratio (I/Glg) was estimated by the following formula:

$$(ISR(t)/hPF + [I(t)]) / \{ [Glg(t)] * (1 + MCR_{Glg}/hPF) \}$$

where $ISR(t)$ is the insulin secretion rate at time t , hPF is hepatic plasma flow; $[I(t)]$ and $[Glg(t)]$ are the measured (peripheral) plasma concentrations of insulin and glucagon at time t ; and MCR_{Glg} is the metabolic clearance rate of glucagon as previously described [26].

β -cell function was quantitated by mathematical modelling of the plasma C-peptide response, as described [28]. The model yields estimates of β -cell glucose sensitivity (the average slope of the relationship between insulin secretion rates and plasma glucose concentrations at corresponding times of the MTT), glucose rate sensitivity (the insulin secretory response to the rate of change in plasma glucose concentrations), and total insulin output (IS, the total amount of insulin released over a given time interval [29]).

Analytical Procedures Plasma glucose was measured by the glucose-oxidase technique (Glucose Analyser II, Beckman Analysers), plasma insulin and C-peptide by electro-chemiluminescence (COBAS e411 instrument, Roche, Indianapolis, USA). Plasma triglycerides, free fatty acids (FFA), glycerol and serum high-density lipoprotein (HDL) cholesterol were assayed in duplicate by standard spectrophotometric methods on a Synchron Clinical System CX4 (Beckman Instruments, Fullerton, USA). Plasma GLP-1, GIP and ghrelin were measured using Milliplex® kit (Merck KGaA, Darmstadt, Germany) on Luminex® (Millipore Corporation, Billerica, MA, USA). Plasma glucagon was measured using radioimmunoassay (Millipore Corporation, Billerica, MA, USA). Blood samples for C-peptide and insulin were drawn using a

protease inhibitor (5 µl/ml of 10 mg/ml of gabexate mesilate); for GLP-1, GIP, ghrelin, and glucagon measurements, blood was drawn into tubes containing protease inhibitor plus a DPP-IV inhibitor (Diprotin A, 10 mM, Sigma Aldrich, Saint Louis, MO, USA). No significant cross-reaction between hormones (GLP-1, GIP, and glucagon) was declared by the manufacturer. Tracer enrichments were measured by gas chromatography/mass spectrometry (GCMS 5975 Agilent Technologies, Fullerton, CA USA) as described previously [16, 25].

Statistical analysis Data are given as the mean±SE or, for variables with a skewed distribution, as the median and [interquartile range]. Integration of area under curves (AUC) was performed by the trapezium rule; mean rates over specified time intervals were then obtained as the ratio of AUC to time interval. Group comparisons were carried out by ANOVA, χ^2 or Mann-Whitney U test, for continuous, nominal, and non-normally distributed continuous variables, respectively. Time-related changes in variables by treatment group were tested by 2-way ANOVA for repeated measures (for these analyses, non-normally distributed variables were log-transformed); when the group \times treatment term was statistically significant, the p value for the treatment effect was not reported.

Results

At baseline, the exenatide (EXE) and the control group (CT) were well matched for gender, age, BMI, and the main clinical and laboratory characteristics (except for small differences in HDL-cholesterol and ALT levels) (**Supplemental Table 1**). The patients did not have any sleep alterations or use of hypnotic drugs nor did they report sleep modification following 3 month of treatment. Exenatide-treated patients experienced mild-to-moderate nausea during the first few weeks of treatment, but in no patient was it necessary to stop the medication (**Supplemental Table 2**). After three months, in the EXE group BMI, waist circumference, and fat mass were significantly reduced (BMI change $-5.5\pm1.0\%$ vs $-2.2\pm1.3\%$ of the CT

group, $p=0.04$); this weight loss was accompanied by a significant reduction in serum γ GT levels (Supplemental Table 1).

Fasting plasma glucose concentrations were similar in the two groups at baseline and did not change at study close. On the MTT, mean glucose concentration was significantly lower in EXE than CT (Supplemental Table 1). The time-course of plasma glucose levels (**Figure 1**) was altered by exenatide, with significantly reduced excursions between 60-180 min after meal ingestion ($AUC = 809 \pm 21$ mmol/L·h falling to 663 ± 7 vs 849 ± 28 to 833 ± 26 in CT, $p=0.03$ for the group \times treatment interaction), and a catch-up phase between 180-360 min. This change reflected the significantly reduced rate of oral glucose appearance (RaO) during the 2nd and 3rd hour postmeal (**Table 1**), resulting in oral glucose still appearing at the end of 6 hours (**Figure 2**) without any difference in the total amount of oral glucose recovered in the systemic circulation. The time-courses of plasma insulin and C-peptide concentrations basically mirrored that of plasma glucose levels. In EXE, mean plasma insulin between 60-180 min decreased from 559 ± 81 to 287 ± 60 pmol/L ($p=0.03$ vs the corresponding change in CT, 525 ± 80 to 499 ± 58 pmol/L), and rose thereafter to similar levels in the two groups.

Fasting EGP was similar in the two groups and did not change over time in either. In CT, EGP was markedly suppressed over the first 2 hours postmeal and returned towards baseline thereafter; rates and pattern were maintained at study close. After EXE treatment, following an initial ~60-min suppression EGP rose higher than at baseline between 60-180 min postmeal, then remained higher, such that total AUC was not different from baseline or from CT (Table 1 and **Figure 2**). Over the 60-180-min postmeal interval, insulin-mediated glucose clearance (MCR_G/I) was unchanged in the CT group whereas it was almost doubled following EXE treatment (Table 1). Rates of glycerol appearance were similar across group and treatment both at fasting and following meal ingestion (Table 1).

Fasting insulin secretion rates were similar across group and treatment. Following meal ingestion, insulin release was lower in EXE than CT between 0-180 min and higher during the subsequent 180 min,

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such that total insulin output was not different between groups (**Table 2**). Both whole-body insulin sensitivity during the MTT (as the OGIS index) and the hepatic insulin resistance index (HIR) improved with EXE vs CT by 15-17%. Likewise, insulin sensitivity of lipolysis (as AT-IR) was significantly improved only in EXE group (Table 2). No relationship was found between the changes in insulin sensitivity (as the OGIS index) and the weight loss ($p=ns$).

Fasting plasma insulin decreased significantly after EXE in comparison with placebo, whereas fasting levels of plasma glucagon, GLP-1, GIP, and ghrelin were similar across group and treatment (**Supplemental Table 2**). During the MTT, the incremental responses of glucagon, GLP-1, and GIP were all reduced between 60-180 min (**Figure 3**) (although full statistical significance was only reached for glucagon and GIP, $p<0.05$). The calculated prehepatic insulin-to-glucagon molar concentration ratio (I/Glg) rose ~4 fold from baseline during the first half of the MTT; this change, was greater in EXE vs CT during the second half of the test. Plasma FFA levels were higher in EXE vs CT between 60-180 min after meal ingestion (Supplemental Table 2).

Discussion

In our group of obese subjects with normal glucose tolerance, three months of exenatide treatment (injected twice daily) led to the loss of an average of 7 kg of body weight, mostly accounted for by a reduction in fat mass, in line with previous findings [30]; the treatment was safe and well tolerated. The main metabolic consequences of exenatide treatment were a reduction in the glucose response to a mixed meal and an improvement in insulin sensitivity as compared to participants receiving placebo. The tracer data demonstrated the mechanism of this metabolic adaptation. On exenatide, the rate of appearance of oral glucose in the systemic circulation was clearly reduced between 2-3 hours following meal ingestion, very likely reflecting the slowing down of gastric emptying (Figure 2) [31, 32]. This resulted in lower glucose levels (Figure 1) over that time interval; in turn, plasma insulin and C-peptide levels and insulin secretion

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rates were reduced, confirming that plasma glucose is the primary secretory stimulus under all physiological circumstances. The glucagon response to the meal also was blunted – an expected effect of exenatide – but proportionally less than the insulin response. Consequently, the prehepatic insulin-to-glucagon molar ratio tended to be lower during the first half of the meal absorption. As this ratio is the principal control of hepatic metabolism, endogenous glucose release was less suppressed – in comparison with the control group – during the first half of the test (Figure 2).

Plasma concentrations of insulinotropic hormones, GLP-1 and GIP, also were decreased during the postprandial time interval when appearance of oral glucose was lower, indicating that the rate of intestinal glucose absorption *per se* is the main stimulus for their release. It is interesting to recall that, following gastric bypass surgery accelerated transfer of gastric content to lower gut segments is associated with the reverse pattern, namely, an earlier and higher plasma glucose peak and an enhanced GLP-1 and glucagon responses [26]. Importantly, however, with exenatide treatment all the changes in glucose fluxes, hormones, and substrates were confined within the first half of the postprandial period and reversed over the second half, such that the corresponding values for the entire postabsorptive 6 hours were essentially unaffected. This explains our current finding, that all 3 parameters of β -cell function were not changed by 3 months of active treatment. It must be observed, however, that our subjects were not diabetic by selection, hence their β -cell function was normal despite the obesity. In patients with T2D, the antihyperglycaemic effect of chronic exenatide treatment has been shown to be due to improved β -cell function [5, 9, 33] as well as reduced EGP [10].

Exenatide has been shown to improve cerebral glucose metabolism (CMR_{glu}) in multiple brain areas involved in the regulation of glucose homeostasis and reward [34]. Recently, Daniele et al showed that during the first 2 hours following glucose ingestion the reduced amount of glucose absorbed after exenatide was inversely associated with CMR_{glu} [35]. Thus, some of the metabolic effects here observed might be related to the cross-talk between gut and brain not only through the well known anorexigenic effects [36] but

also through the modulation of gastric absorption [35]. Furthermore, liraglutide has been shown to change the composition of gut microbiota [37], which might be an additional modulation underlying the effects of exenatide.

In high-fat-fed rats [38] and in patients with type 1 diabetes [39], high-dose exenatide has been reported to improve insulin sensitivity. In early T2D patients, 10 µg exenatide twice daily was associated with improved OGIS and Hep-IR independent of changes in body weight [6]. In non-diabetic obese subjects, intravenous GLP-1 augmented insulin-mediated glucose uptake [40]. In the current study, insulin sensitivity was not directly measured (*i.e.*, using the clamp technique). However, fasting (HIR and AT-IR) and meal-derived (OGIS) indices of insulin sensitivity consistently indicated improved sensitivity to endogenous insulin of EGP, lipolysis, and glucose uptake following exenatide treatment. Mechanisms for this effect could be the weight loss and the lower postmeal glucose levels. With regard to the latter mechanism, recent work has analyzed the separate influence of insulin and glycaemia on glucose clearance [41], thereby establishing that higher glucose levels at any given plasma insulin concentration reduce glucose clearance. In accord with this paradigm is the current finding of a significant increase in the ratio of glucose clearance to insulin levels (MCR_G/I) during the same postprandial time interval when glycemic excursions were blunted by exenatide treatment (Table 1). An effect of weight loss on the improvement of insulin sensitivity indices, after exenatide treatment cannot be excluded; in our patients we failed to find a relationship between weight change and insulin sensitivity improvement.

The orexigenic hormone, ghrelin, is secreted from the stomach immediately before the meal, and its release is regulated by nutrient ingestion in a reciprocal time-course to GLP-1. This has led to the hypothesis that ghrelin might influence GLP-1 secretion [42]; on the reverse side, that GLP-1 may contribute to the postprandial suppression of ghrelin. Administration of exendin-4 in rats determines considerable reduction of fasting levels of ghrelin [43]. In human studies using supraphysiological GLP-1 infusions, the late postprandial increase in ghrelin levels was attenuated, possibly due to the higher insulin levels [44]. In the

present study, exenatide had no effect on ghrelin levels during the six hours postmeal, in agreement with observations made in a study of patients with Prader-Willi syndrome [45].

With regard to lipolysis, in EXE fasting rates of glycerol appearance were unchanged in absolute value but significantly reduced *viz.* the fasting plasma insulin concentration (*i.e.*, a lower AT-IR index). With the entry of the meal lipids, rates of lipolysis were unchanged and not different in EXE *vs* CT despite lower levels of insulin after exenatide treatment, suggesting an improvement of postprandial antilipolytic effect of insulin.

A limitation of the study is that on the study day exenatide was administered before the meal, so we cannot differentiate between acute and chronic effects of the drug. Presumably, the effect on gastric emptying and the attendant metabolic changes are short-lived and renewed at each administration (16), whereas at least some of the changes in fasting parameters are likely chronic effects. This was confirmed in one of our subjects in the EXE group in whom the post-treatment study was done twice, with or without exenatide injection on the study day: without exenatide, there was no delay in the appearance of oral glucose in the systemic circulation (data not shown).

In conclusion, in morbidly obese non-diabetic subjects, three months of treatment with exenatide affects the handling of a mixed meal by significantly delaying oral glucose appearance, very likely as a consequence of delayed gastric empty. This shift in oral glucose absorption – with no change in the amount of oral glucose eventually recovered in the systemic circulation – is sufficient to smooth out the plasma glucose excursions, resulting in better glucose tolerance. Plasma insulin concentrations and insulin secretion rates follow a similar pattern, whereby endogenous glucose production is less suppressed with exenatide than placebo over the 2nd and 3rd hour postmeal. These changes occur concomitantly with clinically significant weight loss (~6 kg over 3 months) and without any impairment of β -cell function. In addition, exenatide treatment is associated with an improvement in hepatic, adipose tissue, and whole body insulin sensitivity.

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Legend to the Figures

Figure 1 Plasma glucose (A), insulin (B), and C-peptide concentrations (C) in the control (*left*) and in Exenatide group (*right*) at baseline (Pre) and following 3 months of treatment (Post). Data are mean \pm SEM.

Figure 2 Rate of appearance of oral glucose (RaO) (A), endogenous glucose (EGP) (B), and glycerol (RaGly) (C) in the control (*left*) and in Exenatide group (*right*) at baseline (Pre) and following 3 months of treatment (Post). Data are mean \pm SEM.

Figure 3 Plasma GLP-1 (A), GIP (B), ghrelin (C), and glucagon (D) in the control (*left*) and in Exenatide group (*right*) at baseline (Pre) and following 3 months of treatment (Post). Data are mean \pm SEM.

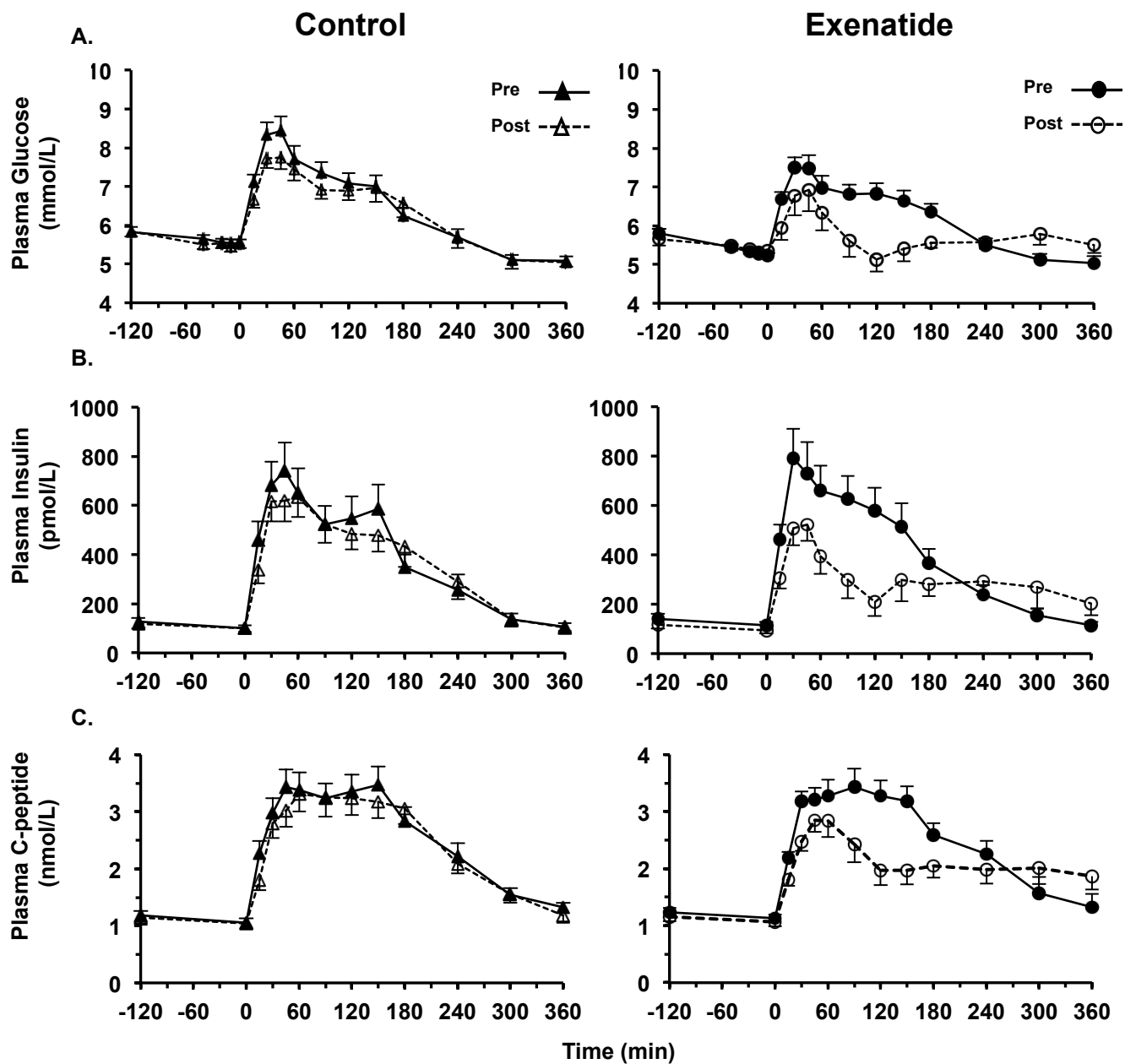


Figure 1

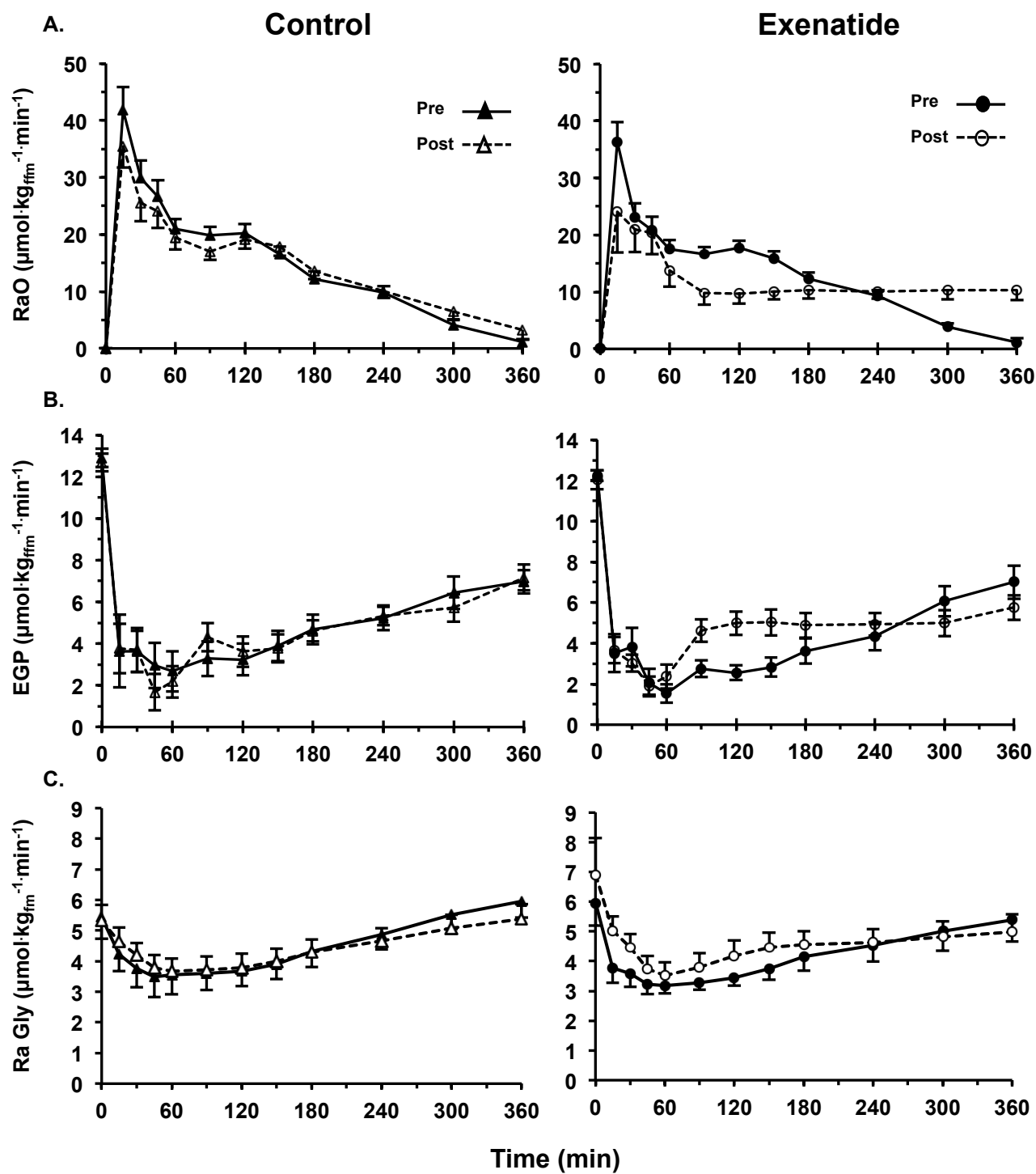


Figure 2

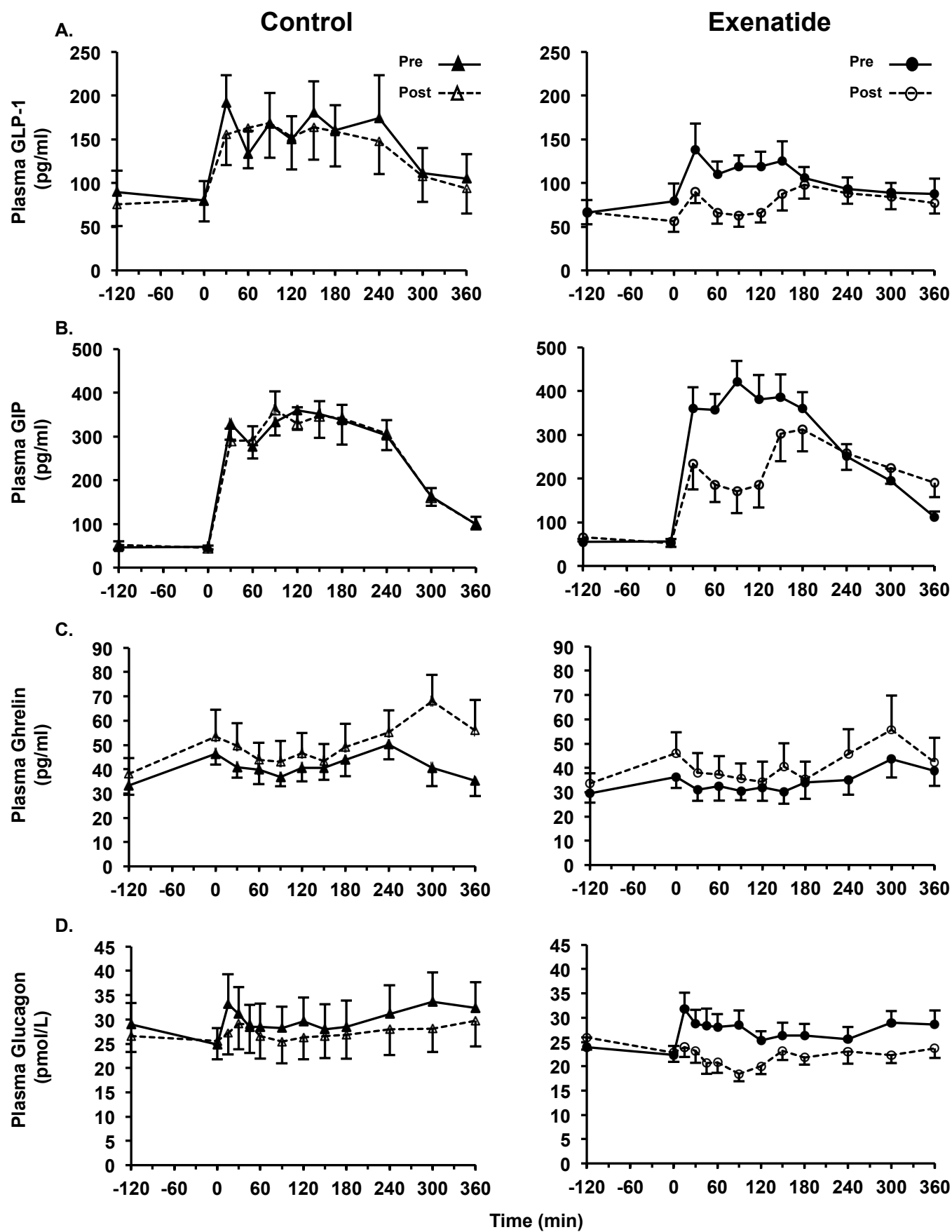


Figure 3

Table 1 – Substrate fluxes.*

	Control		Exenatide		<i>p1</i>	<i>p2</i>	<i>p3</i>
	Pre	Post	Pre	Post			
RaO (60-180) ($\mu\text{mol}\cdot\text{kg}_{\text{ffm}}^{-1}\cdot\text{min}^{-1}$)	2155 [732]	2159 [552]	2002 [734]	1071 [1160]	0.002	-	0.03
RaO (180-360) ($\mu\text{mol}\cdot\text{kg}_{\text{ffm}}^{-1}\cdot\text{min}^{-1}$)	1115 [716]	1439 [892]	1248 [263]	1811 [1255]	ns	0.006	ns
RaO (0-360) ($\mu\text{mol}\cdot\text{kg}_{\text{ffm}}^{-1}\cdot\text{min}^{-1}$)	4825 [1258]	4921 [1468]	4646 [1038]	4449 [2399]	0.04	Ns	ns
Fasting EGP ($\mu\text{mol}\cdot\text{kg}_{\text{ffm}}^{-1}\cdot\text{min}^{-1}$)	13.0 [1.6]	12.5 [1.0]	11.9 [1.2]	11.7 [1.1]	ns	Ns	ns
EGP (60-180) ($\mu\text{mol}\cdot\text{kg}_{\text{ffm}}^{-1}\cdot\text{min}^{-1}$)	415 [314]	402 [223]	333 [197]	576 [362]	ns	0.04	ns
EGP (180-360) ($\mu\text{mol}\cdot\text{kg}_{\text{ffm}}^{-1}\cdot\text{min}^{-1}$)	1168 [444]	1104 [395]	813 [344]	835 [365]	ns	Ns	ns
EGP (0-360) ($\mu\text{mol}\cdot\text{kg}_{\text{ffm}}^{-1}\cdot\text{min}^{-1}$)	1782 [760]	1706 [696]	1324 [789]	1660 [712]	ns	Ns	ns
MCR _{G/I} (60-180) ($\text{ml}\cdot\text{kg}_{\text{ffm}}^{-1}\cdot\text{min}^{-1}\cdot[\text{nmol/L}]^{-1}$)	8.5 [5.4]	8.9 [5.2]	6.4 [4.7]	11.8 [18.7]	ns	-	0.005
MCR _{G/I} (180-360) ($\text{ml}\cdot\text{kg}_{\text{ffm}}^{-1}\cdot\text{min}^{-1}\cdot[\text{nmol/L}]^{-1}$)	15.3 [15.0]	15.5 [19.5]	11.3 [11.8]	13.4 [10.2]	ns	Ns	ns
MCR _{G/I} (0-360) ($\text{ml}\cdot\text{kg}_{\text{ffm}}^{-1}\cdot\text{min}^{-1}\cdot[\text{nmol/L}]^{-1}$)	9.9 [6.8]	10.0 [9.4]	8.5 [5.8]	9.2 [10.2]	ns	Ns	ns
Fasting Ra _{Gly} ($\mu\text{mol}\cdot\text{kg}_{\text{fm}}^{-1}\cdot\text{min}^{-1}$)	4.5 [3.0]	5.1 [2.2]	5.3 [2.0]	5.5 [2.5]	ns	Ns	ns
Ra _{Gly} (60-180) ($\mu\text{mol}\cdot\text{kg}_{\text{fm}}^{-1}\cdot\text{min}^{-1}$)	377 [232]	414 [227]	454 [222]	472 [273]	ns	Ns	ns
Ra _{Gly} (180-360) ($\mu\text{mol}\cdot\text{kg}_{\text{fm}}^{-1}\cdot\text{min}^{-1}$)	839 [420]	879 [378]	794 [572]	781 [353]	ns	Ns	ns
Ra _{Gly} (0-360) ($\mu\text{mol}\cdot\text{kg}_{\text{fm}}^{-1}\cdot\text{min}^{-1}$)	1455 [771]	1489 [732]	1497 [976]	1502 [849]	ns	Ns	ns

* EGP = endogenous glucose production; RaO = rate of oral glucose appearance; Ra_{Gly} = rate of glycerol appearance; MCR_{G/I}= ratio of glucose clearance to plasma insulin levels; in parenthesis are the time intervals over which variables were calculated; data are median [interquartile range]; *p* values are from ANOVA for repeated measures: *p1*= group; *p2* = treatment; *p3* = group \times treatment.

Table 2 – Insulin secretion, insulin sensitivity, and β -cell function.*

	Control		Exenatide				
	Pre	Post	Pre	Post	<i>p1</i>	<i>p2</i>	<i>p3</i>
Fasting ISR ($\text{pmol min}^{-1} \cdot \text{m}^{-2}$)	118 [30]	115 [29]	129 [77]	126 [59]	ns	ns	ns
Insulin output (0-180) ($\text{pmol min}^{-1} \cdot \text{m}^{-2}$)	422 [150]	406 [217]	372 [100]	283 [161]	ns	-	0.02
Insulin output (180-360) ($\text{pmol min}^{-1} \cdot \text{m}^{-2}$)	167 [94]	189 [111]	183 [89]	227 [139]	ns	-	0.05
Total insulin output ($\text{pmol min}^{-1} \cdot \text{m}^{-2}$)	286 [69]	278 [150]	272 [83]	278 [117]	ns	ns	ns
HIR ($\text{mmol kg}_{\text{ffm}}^{-1} \cdot \text{min}^{-1} [\text{pmol/L}]$)	1.23 ± 0.16	1.29 ± 0.20	1.40 ± 0.18	1.16 ± 0.18	ns	-	0.01
AT-IR ($\text{mmol min}^{-1} [\text{nmol/L}]$)	26 [27]	28 [25]	32 [35]	30 [22]	ns	-	0.04
OGIS ($\text{ml min}^{-1} \cdot \text{m}^{-2}$)	316 ± 11	308 ± 13	310 ± 10	359 ± 11	ns	-	0.002
β -cell glucose sensitivity ($\text{pmol min}^{-1} \cdot \text{m}^{-2} \cdot \text{mM}^{-1}$)	115 [87]	108 [40]	142 [68]	129 [85]	ns	ns	ns
Rate sensitivity ($\text{nmol m}^{-2} \cdot \text{mM}^{-1}$)	1.7 [1.4]	1.0 [0.8]	2.0 [1.2]	1.87 [1.3]	ns	ns	ns
Potential factor (fold)	1.14 [0.42]	1.27 [0.45]	1.05 [0.43]	1.17 [0.40]	ns	ns	ns

* ISR = insulin secretion rate; HIR = hepatic insulin resistance index; OGIS = insulin sensitivity; HIR = hepatic insulin resistance index; AT-IR = adipose tissue insulin resistance index; in parenthesis are the time intervals over which variables were calculated; data are median [interquartile range]; *p* values are from ANOVA for repeated measures: *p1*= group; *p2* = treatment; *p3* = group \times treatment.